DIFFERENTIAL TRANSCRIPTION OF T_4-DNA BY DNA-DEPENDENT RNA-POLYMERASE OF E. COLI AND A. NIDULANS

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1. Introduction

It has been shown that bacterial DNA-dependent RNA-polymerases bind to a certain number of specific binding sites on the DNA which is characteristic for the nature of the template [1–4]. These binding sites could either be all of the same type or they could fall into different classes. Polymerases from different sources could either all recognize the same binding sites or different enzyme specific binding sites or different fractions of a number of classes of binding sites.

The next step in the sequence of events involved in the synthesis of RNA is chain initiation. Are all binding sites necessarily also initiation sites? If not, is it possible to differentiate between structural features required for binding and initiation and may these be different for different enzymes?

As a contribution to an answer to these questions recognition of binding sites and initiation by two different enzymes, the RNA polymerases from *E. coli* and from the blue green alga *Anacystis nidulans* are quantitatively compared in this paper.

2. Materials and methods

Preparation and assay of *E. coli* enzyme are described in ref. [5], those of polymerase from *A. nidulans* in ref. [6]. γ-^{32}P-ATP was prepared by the method of Chappell and Glynn [7]. ^{3}H-labeled substrates were purchased from Amersham, Radiochemical Centre, England. DNA was prepared as described previously [8]. In the technique based on the use of an incomplete mixture of substrates each 0.25 ml incubation mixture *assay* was diluted after incubation at 37°C with 1 ml of cold buffer (0.01 M Tris, 0.1 M sodium pyrophosphate, 200 μg albumin/ml, pH 8.0), and filtered through a Millipore filter, which had been soaked with buffer at least 2 hr before use. The filters were washed with 5 ml of the buffer and 90 ml of 0.01 M Tris, 0.1 M Na-pyrophosphate, dried and counted in a liquid scintillation counter. Hybridisation was performed as described in ref. [9].

3. Results and discussion

The quantity of binding sites on T_4-DNA for polymerases from *E. coli* and *A. nidulans* was determined: (1) by saturation of a constant amount of enzyme with increasing amounts of DNA under standard test conditions [6]; (2) by the membrane filter technique of Jones and Berg [2] and (3) by saturation of a constant amount of DNA with increasing amounts of enzyme under conditions which allow initiation but not RNA synthesis, that is in the absence of one of the four substrates (CTP). After preincubation for 3–5 min at 37°C, which allows binding and initiation (formation of short oligonucleotide sequences), heparin is added together with the missing substrate. Consecutive initiation by excess enzyme and reinitiation are blocked completely by this polyanion [10]. The incorporation during the following incubation period is proportional to the number of polymerase molecules bound (and/or initiated) within the preincubation period. When activity is plotted against amount of enzyme present during preincubation a

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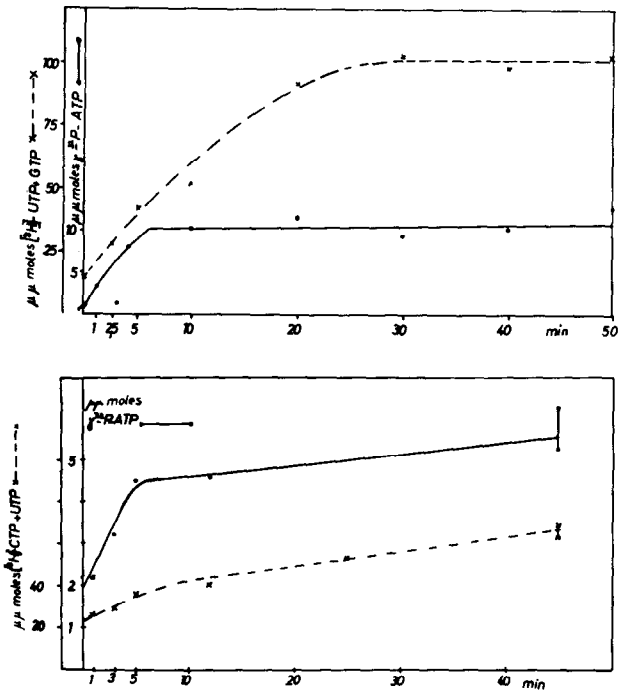

Fig. 1. Kinetics of initiation of RNA-synthesis by (a) E.C.- and (b) A.n.-enzyme (x – – – – x) indicate the quantity of incorporated UTP and GTP respectively. CTP (●——●) indicates the quantity for the 5′ ends.
(a) 35 μg enzyme, 35% active, 21 μg DNA. } 400 mU =
(b) 30 μg enzyme, 34% active, 30 μg DNA. } 100% active

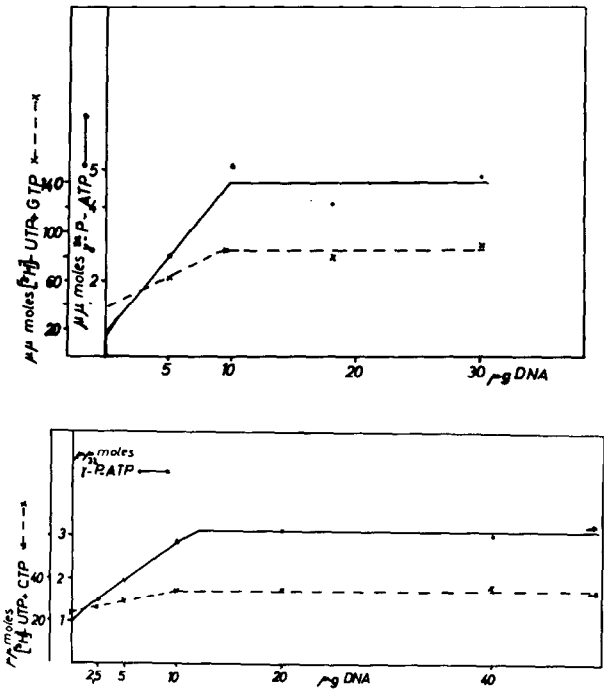

Fig. 2. Saturation of 18 μg (a) E.c. enzyme and (b) A.n. enzyme, with increasing amounts of T_4-DNA. The curve below shows the incorporation of γ-^{32}P-ATP. Incubation time 30 min.

linear increase of activity bends sharply into a plateau so that the saturation point can be determined rather accurately [11].

With all three methods the same number of binding sites for both enzymes, namely one per 2000 nucleotide pairs, was found on T_4-DNA.

This, however, is not sufficient evidence for the assumption that both enzymes bind to the *same* sites.

In an experiment using the "heparin-saturation technique" it has been shown, that successive preincubation of T_4-DNA with excess *E. coli* enzyme and then with A.n. * polymerase does not lead to additional incorporation over that obtained with E.c. enzyme alone within the incubation period with all substrates in the presence of heparin. Therefore, it may be concluded that A.n. enzyme cannot bind to T_4-DNA saturated with E.c. enzyme. The binding sites for both enzymes are indeed the same.

Initiation was measured using a technique which again avoids consecutive binding. Enzyme and DNA were incubated with an incomplete mixture of substrates, among which ATP was labelled with ^{32}P in the γ-position, GTP and UTP were labelled with ^{3}H. 70% of the 5′ ends of RNA synthesized on T_4-DNA by E.c. polymerase [12] and 63% those synthesized by A.n. polymerase [13] are pppAp residues. The difference is not significant. The ratio of ^{32}P to ^{3}H

* *Abbreviations:*
E.c. polymerase: DNA-dependent RNA-polymerase from *E. coli*
A.n. polymerase: DNA-dependent RNA-polymerase from *Anacystis nidulans*
E.c.-RNA: RNA synthesized *in vitro* by polymerase of *E. coli*
A.n.-RNA: RNA synthesized *in vitro* by polymerase of *Anacystis nidulans*

incorporation in the plateau of kinetics (fig. 1a,b) obtained after 5–6 min allows one to calculate that the average chain length of sequences manufactured by both enzymes is 6–7, not allowing a displacement of the enzyme from the binding site sufficient for a second initiation event. The number of RNA chains initiated is the same as the number of ^{32}ppp ends bound to the DNA by enzyme molecules. Since a complex of DNA, enzyme and RNA or oligonucleotides in contrast to free enzyme, RNA or oligonucleotides, is bound by millipore filters from neutral buffer this mixture may be determined directly by a filter technique (see methods). When this number for a constant amount of enzyme is measured with increasing amounts of DNA (incubation time 30 min) a curve is obtained which bends sharply into a plateau at a DNA concentration saturating the given amount of enzyme. From the ratio of the number of 5′ ends measured in DNA saturation (fig. 2a,b) to the saturation amount of DNA the number of initiation points for E.c. enzyme has been found to be 1 per 2000 nucleotide pairs in contrast to 1 per 4000 for A.n. enzyme. Thus, every binding site is also an initiation site for E.c. enzyme whereas only half of the binding sites are efficient initiation sites for A.n. polymerase.

The absolute number of binding sites determined by the technique used depends on an assumption concerning the purity of the enzymes. The equality of the numbers for both enzymes, however, is secured by the fact that DNA saturated with one enzyme no longer binds the other (see above). The twofold difference in the ratio of initiation to binding sites between E.c. and A.n. enzyme is also independent of enzyme purity.

From the kinetics of initiation by A.n. enzyme it may be seen that a slow further initiation follows the fast first phase, whereas a plateau is reached for E.c. enzyme after the same time.

By hybridisation competition experiments with ^{14}C-RNA produced by both polymerases on T_4-DNA and cold RNA produced with the same enzymes on the same DNA it could be shown (fig. 3), that all of the RNA synthesized by A.n. enzyme is displaced by RNA manufactured by E.c. enzyme. The same amount of RNA synthesized by E.c. enzyme is displaced by A.n.-RNA with less than half the efficiency (slope) as in the reverse pairing.

This is a quantitative difference. Qualitatively it

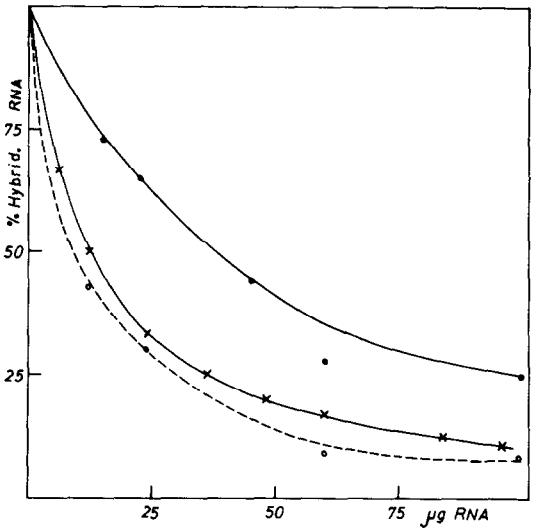

Fig. 3. Competitive hybridisation of 12 µg A.n.-^{14}C-RNA with increasing amounts of unlabeled E.c.-RNA (○----○) and of E.c.-^{14}C-RNA with increasing amount of unlabeled A.n.-RNA (●——●). x——x is the theoretical curve for competitive hybridisation of complete identical species. Hybridisation for 6 hr at 65°C in 2 X SSC.

appears possible to compete almost fully also for E.c.-RNA with high quantities of A.n.-RNA.

The conclusion is, that the transcription products of both polymerases are largely identical. At most, a small fraction of the RNA species produced by E.c. enzyme is absent or very rare in the A.n.-RNA. The fraction of different species of RNA in the products of the two enzymes may also be different quantitatively. From the kinetics of initiation by A.n. enzyme it may be concluded, that a fraction close to 50% of binding sites, which are normal initiating sites for the E.c. enzyme are very inefficient, but definitely active initiating sites for the A.n. polymerase. Therefore, the result of the hybridisation experiment may be explained by the assumption, that the species produced by initiation from these sites are present in low quantity in the transcription product of A.n. polymerase.

Since the *in vitro* transcription product of E.c. polymerase on T_4-DNA is early T_4-RNA, it is clear, that A.n. enzyme in vitro also transcribes only early T_4 genes.

In summary, it can be stated, that the structured requirements for binding and initiating on the DNA may be differentiated. In the case investigated here,

E.c. enzyme is able to utilize all its binding sites for efficient initiation, whereas A.n. enzyme, which also binds to all of these sites, differentiates between two classes of initiation sites, one quite efficient, the other almost inactive. A similar observation has been made in our laboratory in comparing the functions of normal E.c. polymerase and the modified enzyme isolated from T_4-infected *E. coli* cells. In this case, the modified enzyme binds to the same sites on T_4-DNA as the normal enzyme, but is quite inefficient in initiation from these sites. On the other hand, on other templates, such as calf thymus or T_3-DNA, both enzymes initiate and work with similar efficiencies. This means, that binding sites are not necessarily initiation sites, that initiation sites may be different from each other and specific in their relations to different polymerases.

Acknowledgements

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References

[1] J.P.Richardson, J. Mol. Biol. 21 (1966) 83.
[2] O.W.Jones and P.Berg, J. Mol. Biol. 22 (1966) 199.
[3] H.Bremer, M.Konrad and R.Bruner, J. Mol. Biol. 16 (1966) 104.
[4] N.Sternberg and A.Stevens, Biochem. Biophys. Res. Commun. 24 (1966) 937.
[5] W.Zillig, E.Fuchs and R.L.Millette, in: Progress in Nucl. Acid Res., eds, G.L.Cantoni and D.R.Davies (Harper and Row, New York, 1966).
[6] K.v.d.Helm and W.Zillig, Hoppe-Seyler's Physiol. Chem. 348 (1967) 902.
[7] J.M.Glynn and J.B.Chappell, Biochem. J. 90 (1964) 147.
[8] H.Schuster, G.Schramm and W.Zillig, Z. Naturforsch. 11b (1964) 339.
[9] D.Gillespie and S.Spiegelmann, J. Mol. Biol. 12 (1965) 829.
[10] G.Walter, W.Zillig, P.Palm and E.Fuchs, European J. Biochem. 3 (1967) 194.
[11] W.Zillig, manuscript in preparation.
[12] H.Bremer, M.W.Konrad, K.Gaines and G.S.Stent, J. Mol. Biol. 13 (1965) 540.
[13] K.v.d.Helm, unpublished results.